

Measurement of Chlorophyll *a* and Carotenoids Concentration in Cyanobacteria

Tomáš Zavřel^{1*}, Maria A. Sinetova² and Jan Červený¹

¹Department of Adaptation Biotechnologies, Global Change Research Centre, Academy of Science of the Czech Republic, Drásov, Czech Republic; ²Laboratory of Intracellular Regulation, Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia

*For correspondence: zavrel.t@czechglobe.cz

[Abstract] This is a protocol for precise measurement of chlorophyll *a* and total carotenoid concentrations in cyanobacteria cells. Cellular chlorophyll concentration is one of the central physiological parameters, routinely followed in many research areas ranging from stress physiology to biotechnology. Carotenoids concentration is often related to cellular stress level; combined pigments assessment provides useful insight into cellular physiological state. The current protocol was established to minimize time and equipment requirements for the routine pigments analysis. It is important to note that this protocol is suitable only for cyanobacteria containing chlorophyll *a*, and is not designed for species containing other chlorophyll molecules.

Materials and Reagents

1. Cyanobacteria culture (Note 1)
2. Methanol ≥99.9% (GC) (Sigma-Aldrich)

Equipment

1. Eppendorf safe-lock tubes (1.5 ml)
2. Centrifuge with relative centrifugal force (RCF) of 15,000 x *g* and cooling option to +4 °C (Sigma-Aldrich, model: 1-16 K)
3. Pipette 100 µl -1,000 µl + pipette tips (RAININ, Mettler-Toledo)
4. Fridge (+4 °C)
5. Spectrophotometer with slit width 1 nm (Shimadzu, model: UV-2600)
6. Spectrophotometric plastic or glass VIS/UV-VIS semi-micro 0.75-1.5 ml cuvettes
7. Mixing device (Silamat S6, Ivoclar Vivadent) or vortex (IKA MS3 digital, IKA®)
8. Aluminum foil
9. Holder for Eppendorf tubes

Procedure

1. Work under modest irradiance [up to $5 \mu\text{mol (photons) m}^{-2} \text{s}^{-1}$ of white light or $10 \mu\text{mol (photons) m}^{-2} \text{s}^{-1}$ of green light] in order to prevent degradation of extracted pigments.
2. Harvest 1 ml of cyanobacterial culture suspension (Note 2).
3. Centrifuge cells at $15,000 \times g$ at laboratory temperature for 7 min and thoroughly discard supernatant. If necessary repeat the centrifugation (Note 3).
4. Add 1 ml of methanol, precooled to $+4 \text{ }^{\circ}\text{C}$.
5. Homogenize the sample by mixing (Silamat S6, 2 sec), vortexing (2,000 rpm, 4 sec), or by gentle pipetting up and down.
6. Cover the samples with aluminum foil. Incubate at $+4 \text{ }^{\circ}\text{C}$ for 20 min in order to extract the pigments from the cells (Note 4).
7. Centrifuge at $15,000 \times g$, $+4 \text{ }^{\circ}\text{C}$ for 7 min and visually check pellet; it should be ranging between bluish and purple (Figure 1) with no green color. If the pellet is green, repeat steps 5-6.
8. Calibrate spectrophotometer using methanol as blank.
9. Measure pigments concentration by spectrophotometer with slit width 1 nm.
 - a. Measure absorbance of sample and blank at 470 nm, 665 nm and 720 nm (Note 5).
 - b. Calculate concentration of chlorophyll a content according to equations:

$$\text{Chl}_a [\mu\text{g/ml}] = 12.9447 (A_{665} - A_{720}) \text{ (Ritchie, 2006)}$$

$$\text{Chl}_a [\mu\text{M}] = 14.4892 (A_{665} - A_{720}); \text{ for Chl}_a \text{ molar mass} = 893.4890 \text{ g/mol}$$

$$\text{Carotenoids} [\mu\text{g/ml}] = [1,000 (A_{470} - A_{720}) - 2.86 (\text{Chl}_a [\mu\text{g/ml}])] / 221 \text{ (Wellburn, 1994)}.$$
10. Perform the analysis at least in triplicates as necessary for calculations of averages and standard deviations from each pigments assessment.

Representative data

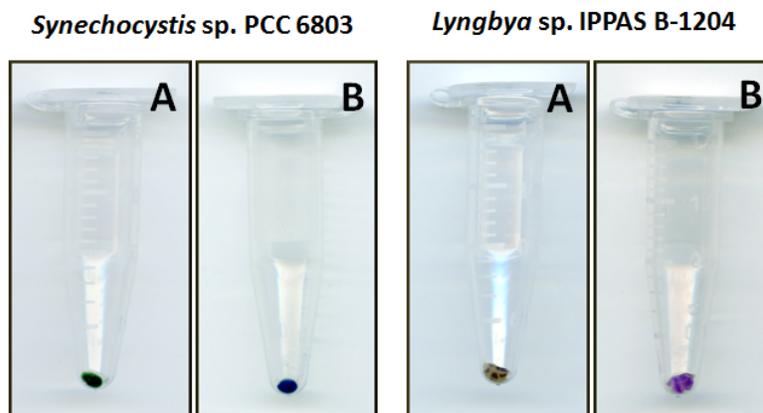


Figure 1. Colors of cyanobacteria pellets before addition of methanol (A) and after chlorophyll a and carotenoids extraction (B). The color of pellets after the methanol extraction will be ranging

from bluish (*Synechocystis* sp. PCC 6803) to purple (*Lyngbya* sp. IPPAS B-1204), depending on particular combination of phycobiliproteins.

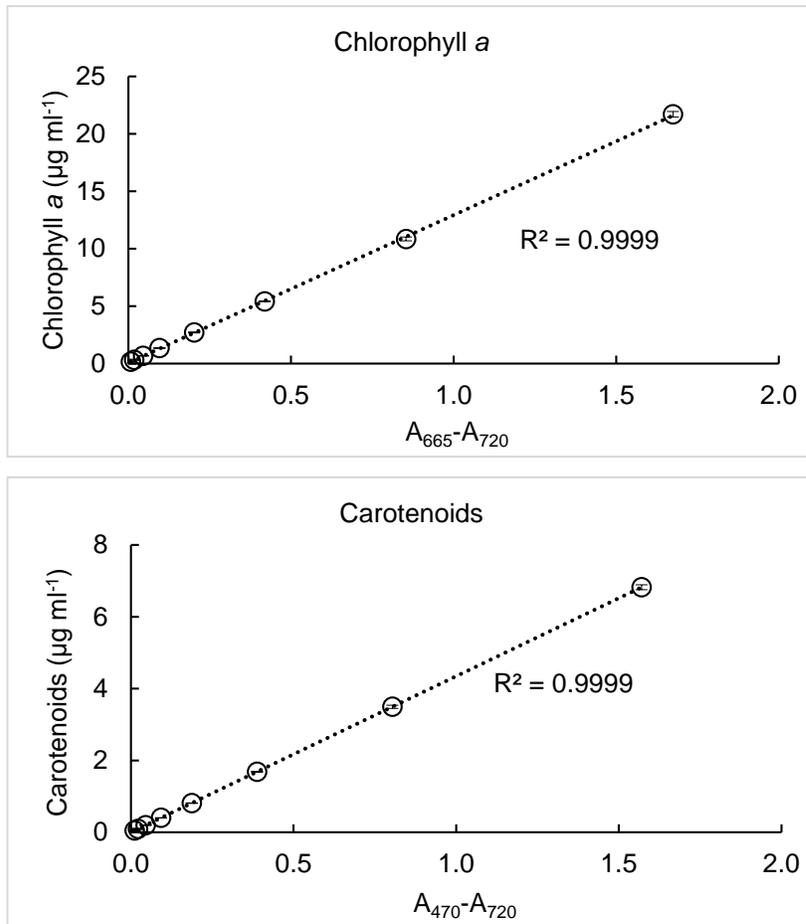


Figure 2. Representative measurements of chlorophyll a and carotenoids concentrations in cell culture of cyanobacterium *Synechocystis* sp. PCC 6803. Dense cyanobacteria culture (chlorophyll a: 22 $\mu\text{g/ml}$, carotenoids: 7 $\mu\text{g/ml}$) was gradually diluted by half up to pigments concentration 0.1 $\mu\text{g/ml}$. Each measurement was performed in triplicates; the error bars represent standard deviations.

Notes

1. The authors are not aware of any restrictions of this protocol usage for cyanobacterial strains containing only chlorophyll a. The protocol was successfully applied to *Cyanothece* sp. ATCC 51142, *Synechococcus elongatus* sp. PCC 7942, *Cyanobacterium* sp. IPPAS B-1200, *Synechocystis* sp. PCC 6803, *Arthrospira platensis* IPPAS B-256, *Anabaena sphaerica* IPPAS B-404, *Chroococcus* sp. IPPAS B-1203, *Lyngbya* sp. IPPAS B-1204, *Aphanocapsa* sp. IPPAS B-1205, *Anabaena* sp. IPPAS B-1206.

2. The amount of cell suspension required for analysis can vary with the culture density. With very diluted cultures it is recommended to harvest bigger culture volume; for cultures with chlorophyll a density around 10 ng(Chl) ml^{culture}⁻¹ or lower even up to 5 ml. On the contrary, for very dense cultures lower sample volume is recommended – with high cellular densities, some pigments can remain in cells after extraction.
3. If some supernatant remains in the tube after the first centrifugation, the extraction will not take place in pure methanol and the pigment concentrations will not be measured properly.
4. The pigment extraction time can be prolonged up to 2 hours with no significant pigment degradation.
5. The final absorbance at each wavelength should be in linear absorbance range. For spectrophotometer UV-2600 (Shimadzu) this linear absorbance range is 0.01 - 2.5. If necessary, dilute the sample with methanol to fit in the spectrophotometer linear absorbance range.

In case of using different volumes of cyanobacteria samples and/or methanol than 1 ml, the final pigment concentration should be calculated according to following equation:

$$\text{Pigment concentration in sample} = \text{measured pigment concentration} \times \frac{\text{volume of methanol (ml)}}{\text{volume of sample (ml)}}$$

Acknowledgements

The protocol was adopted from publication “On the dynamics and constraints of batch culture growth of the cyanobacterium *Cyanothece* sp. ATCC 51142” (Sinetova *et al.*, 2012). T. Z. and J. C. were supported by the MEYS CR within CzechGlobe Centre, reg. no. CZ.1.05/1.1.00/02.0073 484, the National Sustainability Program I (NPU I), grant number LO1415 and by EC OP project, reg. no. CZ.1.07/2.3.00/20.0256. S. M. A. was supported by grant from Russian Scientific Foundation no. 14-24-00020).

References

1. Balizs, G., Benesch-Girke, L., Borner, S. and Hewitt, S. A. (1994). [Comparison of the determination of four sulphonamides and their N4-acetyl metabolites in swine muscle tissue using liquid chromatography with ultraviolet and mass spectral detection.](#) *J Chromatogr B Biomed Appl* 661(1): 75-84.
2. Ritchie, R. J. (2006). [Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents.](#) *Photosynth Res* 89(1): 27-41.
3. Sinetova, M. A., Červený, J., Zavřel, T. and Nedbal, L. (2012). [On the dynamics and constraints of batch culture growth of the cyanobacterium *Cyanothece* sp. ATCC 51142.](#) *J Biotechnol* 162(1): 148-155.

4. Wellburn, A. R. (1994). [The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution.](#) *J Plant Physiol* 144:307–313.